

Please amend the application as follows:

In the Specification

Please replace the paragraph at page 9, line 11 through page 10, line 6 with the following paragraph:

To facilitate the identification of fluorescence insensitive sites which can accommodate the presence of a binding site and/or the production aFPs, an affinity fluorescent protein cassette can be created by introducing a small synthetic test peptide comprising one or more appropriate restriction enzymes sites at candidate locations in the sequence of a fluorescent protein such as GFP. After identifying regions (e.g., guest loops) of the fluorescent protein which can tolerate the introduction of the test peptide without a loss of fluorescent intensity, the restriction sites can be used for the introduction of heterologous amino acid sequences, or non-protein moieties which embody the desired binding site. For example, the hexapeptide LEPRAS (SEQ ID NO: 1) which contains three restriction enzyme sites (XhoI-AvrII-NheI) was useful for identifying fluorescent insensitive sites in the GFP molecule. Alternatively, other test peptides can be designed which exhibit characteristics such as hydrophobicity and charge in common with either the native loop or with the heterologous amino acid sequence or moiety selected for introduction into the fluorescent protein. Affinity and specificity of binding of an aFP of the present invention can be further tailored by additional modification of the same loop, for example, by introducing two or more binding sites (e.g., linear or cyclic peptides) in tandem at a single location, or by introducing the same binding site at distinct locations. For example, two binding sites can be introduced at the position between Gln157 and Lys158 (e.g., 157HA2 or 157HA2) or at the position between Glu172 and Asp173 (e.g., 172HA, 172HA2). Alternatively, a single copy of each binding site can be introduced at two or more distinct sites (e.g., 157HA/172HA). Depending on the nature of the target ligand, affinity of the binding of an aFP of the present invention may also be enhanced by introducing an additional binding sites at either, or both, the N-terminus and C-terminus (e.g., 157/CHA) of the GFP molecule.

Please replace the paragraph at page 16, lines 14 through 19 with the following paragraph:

b2
Expression Vectors. The original plasmid pEGFP purchased from Clontech (accession #U76561). Vector pProEX Hta from Life Technologies (cat.#10711-018) containing (His)6 tag at the amino-terminus for affinity purification. Restriction enzymes and DNA ligases were purchased from New England Biolabs (Beverly, MA). PCRs were performed on ROBOCYCLER GRADIENT 96 (Stratagene) using PCR Supermix (Life Technologies). DNA purification and gel extraction were done using QLAGEN kits.

Please replace the paragraph at page 18, lines 9 through 14 with the following paragraph:

b3
Absorption, Excitation, and Emission Spectra. The absorption spectra were collected on an AVIV Model 118DS spectrophotometer. (AVIV Associates, Inc., Lakewood, NJ) at 25°C. Excitation and emission spectra were recorded on a FLUOROLOG 3-22 spectrofluorimeter (Instruments S.A., Inc., Edison, NJ) at 25°C. The instrument parameters are the following: slit of 2.5 nm, integration time of 0.5 second, interval of 1 nm, and PMT 950V.

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i-ii).

In the Claims

Please amend Claims 11-15. Amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (page iii).

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B4
11. (Amended) An isolated affinity fluorescent protein expression cassette comprising a modified green fluorescent protein (GFP) nucleic acid sequence which is mutated and operatively linked to expression control sequences, wherein the modified GFP sequence comprises a recombinant peptide which comprises restriction endonuclease sites introduced at a location of the GFP molecule selected from the group consisting of: